Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 5 Issue 1 January 2023

Research Article

Characterization of Microsatellite Markers in Crossbred Ducks

Purabi Kaushik[1]*, Raj Jyoti Deka[1], Dishanta Dutta[2], Indu Borah Dutta[2], Mrinal Bora[2], BN Bhattacharya[1] and DC Mili[1]

[1]*College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India*

[2]*Government Duck and Poultry Farm, Joysagar, Sivasagar, Assam, India*

***Corresponding Author:** Purabi Kaushik, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India.

Received: July 11, 2022

Published: Decemeber 08, 2022

© All rights are reserved by

Purabi Kaushik., *et al.*

Abstract

The study was conducted at College of Veterinary Science, Assam Agricultural University, Khanapara, Assam. In this study, the genomic DNA was isolated and characterized microsatellite loci in crossbred duck (*Pati x White Pekin*). Ten microsatellites were used to detect polymorphisms in 50 cross bred ducks. A total of 28 nos of alleles were observed and all loci were polymorphic. Per microsatellite locus number of alleles ranged from 2 to 5 with an average of 2.6 ±0.08. The observed and expected heterozygosity of these polymorphic makers ranged from 0.00 to 0.42 with an average number of 0.047 and 0.41 to 0.82 with an average number of 0.57. The Polymorphic markers consist of observed heterozygosity's of loci were less than 0.50. The polymorphism information content (PIC) of 10 loci ranged from 0.32 to 0.78 with an average of 0.477. Microsatellite markers can be a tool for mitigating the genetic linkage map of the duck as well as comparative mapping with the chicken.

Keywords: Microsatellite Markers; Polymorphism; PIC; Cross Bred (Pati duck White Pekin)

Introduction

The quantitative traits which is controlled by polygene and the molecular genetic mapping tools enables the identification of quantitative trait loci in the genome. Microsatellites were found abundantly and at random throughout most eukaryotic genomes [4]. A large number of microsatellites have been isolated and widely used for these purposes. In case of duck populations less genetic markers have been established. Thus, we attempted to isolate microsatellite markers for cross bred duck and to investigate their polymorphism.

Chicken represents a valuable genetic resource and protein source, it remains a potential threat to human health as they serve as a reservoir for diseases and food borne pathogens. Diversification of the poultry production is one of the viable options to enhance the food production with less susceptibility to the threats from the emerging diseases and changing climatic conditions. Ducks are promising species suitable for the diversification and will be complimentary with an adaption potential from small to commercial farming. Majority of ducks in the country are desi type with a meagre proportion of improved duck strains. Recent livestock census indicated that the duck population has been drastic decreasing over years from 27.6 million in 2007 to 23.5 million in 2012. Therefore, there is an urgent need to augment the duck production through improved breeding, feeding, disease control and other managemental strategies. Further, fine-tuning of production performance and effective health care management can be achieved with the help of newer technologies. Converged use of various conventional molecular and health care technologies will augment duck egg and meat production. Moreover, prevailing ducks are desi or non-descript type having low production potential. There is an urgent need of duck improvement. Besides, Limited research and scientific intervention has been paid to characterize them, to improve their productivity and to exploit their unique characteristics. Hence, any attempt to improve the duck farming will have direct bearing on the economically weaker section of the society. Improvement of productivity of ducks through identification of molecular markers could be a method of choice. Therefore, genetic characterization by developing duck specific microsatellite markers and designing suitable improvement program are re-

quired to be undertaken at the earliest. The microsatellite markers are extensively used for assessing genetic structure, diversity, and relationships. Information about genetic diversity of indigenous duck breeds is important to design effective improvement and conservation strategies. Therefore, the current studies aims at genetic characterization and evaluation of growth, production and reproduction traits of the crosses of local duck population besides undertaking a suitable duck improvement program [1].

Materials and Methods

- **DNA isolation protocol:** About 1.5 mL of distilled water was added to the duck blood (35-50 µL) that was placed in the anticoagulation tube. The content was mixed well and transferred into a 1.5 mL micro centrifuge tube and centrifuged for 2 min at 13500 rpm in a micro centrifuge. The supernatant was discarded, and the repeated the steps. The pellet was resuspended in a a 55°C prewarmed 1 mL of WBC lysis buffer (10 mM Tris-Cl pH7.7, 1.5M NaCl, 2 mM EDTA, 0.5% SDS), and then the whole suspension was mixed well and micro centrifuged at 13500 rpm for 4 min. The supernatant containing DNA thread mass was picked up with a micropipette equipped with a wide orifice and placed in a new tube. To the supernatant, 1 ml of absolute ethanol was added, and the tube was inverted several times. The DNA threads were picked up and placed in a new micro centrifuge tube containing 1 mL of ice cold 70% ethanol and mixed well. Micro centrifugation was performed at 13500 rpm for 4 min. Supernatant was discarded and DNA was resuspended in 0.5 mL of TE buffer.
- **Spectrophotometric evaluation of DNA extraction:** The spectrophotometric evaluation of the concentration and purity of DNA was carried out by spectrophotometer. DNA concentration was evaluated. DNA purity with regards to protein and salt contaminants was based on the A 260/280 and A 260/230 absorbance ratios respectively.
- **The electrophoretic evaluation of DNA extraction:** The genomic DNA integrity was checked electrophoretically in agarose gel.

Microsatellite primers

10 informative duck microsatellite markers were identified from database as reported by [1] and [5] and used for the studies. The synthesized primer pairs were obtained in lyophilized form and were reconstituted with nuclease-free water as per manufacturer's instructions. A stock of 100 µM was prepared and from this working primer solution of 10 pM was prepared and used in PCR.

Sl.	Primer Name	Accession Number	Nucleotide Sequence of Forward (F) and Reverse (R) Primers	T_a (⁰C)	Ref
1.	CAUD001	AY493246	F-GCAGAAAGTGTATTAAGGAAG R-ACAGCTTCAGCAGACTTAGA	54	Alyethodi., *et al.* 2010
2.	CAUD002	AY493247	F-CTTCGGTGCCTGTCTTAGC R-AGCTGCCTGGAGAAGGTCT	60	Alyethodi., *et al.* 2010
3.	CAUD003	AY493248	F-CCTGGCATTCTGCTAAGTTC R-TGGGTTTGAACAGTGTAGCC	51.4	Y. Hung., *et al.* 2005
4.	CAUD004	AY493249	F-TCCACTTGGTAGACCTTGAG R-TGGGATTCAGTGAGAAGCCT	60.8	Y. Hung *etal.*,2005
5.	CAUD005	AY493250	F-CTGGGTTTGGTGGAGCATAA R-TACTGGCTGCTTCATTGCTG	60	Alyethodi., *et al.* 2010
6.	CAUD006	AY493251	F-ATGGTTCTCTGTAGGCAATC R-TTCTGCTTGGGCTCTTGGA	56	Alyethodi., *et al.* 2010
7.	CAUD007	AY493252	F-ACTTCTCTTGTAGGCATGTCA R-CACCTGTTGCTCCTGCTGT	60	Alyethodi., *et al.* 2010
8.	CAUD009	AY493253	F-AGGGATTTTGGAGCGGAGC R-TGTGCGGCGTTTTCCTCTG	63	Y. Hung., *et al.* 2005
9.	CAUD010	AY493254	F-GGATGTGTTTTTCATTATTGAT R-AGAGGCATAAATACTCAGTG	50	Alyethodi., *et al.* 2010
10.	CAUD013	AY493258	F-ACAATAGATTCCAGATGCTGAA R-ATGTCTGAGTCCTCGGAGC	58	Y. Hung., *et al.* 2005

Table 1

Polymerase chain reaction (PCR)

Each PCR assay was carried out in a total of 25 µL containing 12.5 µL PCR master mix (2X), 5.5 µL Nuclease Free Water, 5 µL Template DNA, 1 µL each of reverse and forward primer. Initial denaturation was done at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at optimized temperature for 1 min and extension at 72°C for 1 min and then final extension at 72°C for 5 min. The PCR products were analyzed by Agarose gel electrophoresis [3].

Following PCR cycling conditions were optimized for 10 microsatellite loci

Heat inactivation at 95^{0}C for 5 minutes
30 cycles of

- Denaturation at 94^{0}C for 1 minute,
- Annealing at $T_a{}^0$C (T_a= Optimized annealing temperature for each microsatellite primer pair) for 45 seconds
- Extension at 72^{0}C for 45econds.
  - Final extension at 72^{0}C for 5minutes.
  - 4°C forever

The PCR products were kept at-200C until further analysis

Documentation of PCR products by agarose gel electrophoresis

Approximately,10µl of PCR product was added with 2µl Bromophenol blue dye (6X loading dye, GCC Biotech, India Pvt. Ltd.) for loading in the gel. Samples were loaded into wells of 2% agarose gel containing ethidium bromide (5 µl per 100 ml of 1X TBE buffer) along with 5 µl of100bp DNA ladder (GCC Biotech, India Pvt. Ltd.) as molecular size marker for identification of the desired product. The electrophorese is was done at 2-5volts/cm. The products were examined under UV light in Gel Documentation system (Biorad Laboratories, USA) and documented.

Metaphor agarose gel electrophoresis (MAGE) of microsatellites alleles

The confirmed amplification of all the samples, the amplicons were run on 3% metaphor agarose gel electrophoresis (MAGE) to resolve microsatellite alleles for further genotyping.

Determination of molecular size of microsatellite alleles and genotypes

The molecular sizes (in bp) of all the alleles at ten studied microsatellites were determined with the help of Image Lab software (Bio-Rad Laboratories Inc., U.S.A.) through Gel Doc system. Genotypes of all the birds were determined on the basis of presence of microsatellite alleles.

Statistical analysis of population genetics data

Data on genotype of all experimental birds at eighteen microsatellites were compiled and analyzed using POPGENE® 3.1 software [8] for their population genetics parameters. The primary data on genotype was subjected to co-dominant marker diploid data analysis to estimate observed and expected genotypic frequencies, Hardy-Weinberg (HW) equilibrium status, allele frequency, observed and effective number of alleles, percentage of polymorphic loci, observed and expected homozygosity and heterozygosity, and Shannon index.

Genetic variability analysis

Average heterozygosity per microsatellite marker was calculated according to Nei (1978).

$$H_i = \frac{2N}{2N-1}\left(1 - \sum_{j=1}^{k} P_j^2\right)$$

Where P_j is the frequency of the jth allele at i^{th} locus with k number of alleles in a population and N is the number of individuals, assuming that the population was under Hardy-Weinberg equilibrium.

Polymorphic Information Content (PIC) at each microsatellite locus was calculated using the following formula (Botstein., *et al.* 1980).

$$PIC = 1 - \sum_{i=1}^{k} p_i^2 - \sum_{i=1}^{k-1}\sum_{j=i+1}^{k} 2p_i^2 p_j^2$$

Where

P_i and P_j are the frequencies of ith and j^{th} alleles, respectively at *a* locus with *k* numbers of alleles in the population.

Results and Discussion

The characteristics of the 10 microsatellite loci with allele frequencies were summarized as table 1 and Table 2 respectively. A total of ten microsatellites were used to detect polymorphisms in 50 cross bred ducks. A total of 28 no's of alleles were observed and all loci *were* polymorphic. The number of alleles ranged from 2 to 5 with an average of 2.6 ± 0.08 per microsatellite locus. The observed and expected heterozygosity of these polymorphic makers

ranged from 0.00 to 0.42 with an average number of 0.047 and 0.41 to 0.82 with an average number of 0.57, respectively. Among the polymorphic markers, the observed hetero zygosities of loci were less than 0.50. The polymorphism information content (PIC) of 10 loci ranged from 0.32 to 0.78 with an average of 0.477. Based on the classification of [2,3] five (50%) polymorphic markers were highly informative (PIC > 0.50) and rest five (50%) were reasonably informative (0.50 > PIC > 0.25) which can be comparable to [4,7].

Microsatellite Loci	Allele Number	Size	Alleles	Allele frequency
CAUD001	3	158	A	0.2684
		197	B	0.1650
		220	C	0.5666
CAUD002	2	255	A	0.2307
		278	B	0.7693
CAUD003	3	190	A	0.3076
		210	B	0.1923
		225	C	0.5001
CAUD004	4	185	A	0.0852
		210	B	0.2508
		213	C	0.4314
		228	D	0.2326
CAUD005	5	195	A	0.3461
		215	B	0.2172
		230	C	0.1011
		245	D	0.2116
		263	E	0.124
CAUD006	2	155	A	0.8125
		180	B	0.1875
CAUD007	2	135	A	0.5652
		95	B	0.4348
CAUD009	2	128	A	0.7251
		110	B	0.2749
CAUD010	2	105	A	0.4215
		119	B	0.5785
CAUD013	3	85	A	0.1566
		98	B	0.2175
		101	C	0.6259
Mean ± SE	22.6 ± 0.08			

Table 1: Number of alleles, their molecular sizes and frequencies at various microsatellite loci in crossbred (Crosses between Pati and White Pekin).

Primer Name	PIC	I	n_a	n_e	Nei	Fis	H_o	H_e	Chi square	Probability	G square	Probability
CAUD001	0.53	1.02	3.00	2.69	0.63	1	0.01	0.64	76.75***	0.00001	75.75***	0.000000
CAUD002	0.38	0.71	2.00	1.81	0.49	1	0.00	0.45	38.66***	0.000	46.81***	0.000000
CAUD003	0.57	1.05	3.00	2.65	0.65	1	0.00	0.65	75.76***	0.000	76.74***	0.000000
CAUD004	0.65	1.13	4.00	3.33	0.74	0.80	0.42	0.76	115.04***	0.000012	102.45***	0.000001
CAUD005	0.78	1.52	5.00	4.19	0.80	0.45	0.04	0.82	141.75***	0.000018	107.20***	0.000151
CAUD006	0.33	0.69	2.00	1.68	0.42	1	0.00	0.41	36.66***	0.000	42.55***	0.000000
CAUD007	0.32	0.68	2.00	1.67	0.41	1	0.00	0.41	36.45***	0.000	42.21***	0.000000
CAUD009	0.33	0.68	2.00	1.68	0.42	1	0.00	0.45	36.66***	0.000	42.55***	0.000000
CAUD010	0.33	0.69	2.00	1.67	0.42	1	0.00	0.45	36.66***	0.000	42.55***	0.000000
CAUD013	0.55	1.09	3.00	2.67	0.62	1	0.00	0.66	75.85***	0.000	77.91***	0.000000
Mean ± SE	0.477 ± 0.02	0.926 ± 0.06	2.8 ± 0.02	2.404 ± 0.01	0.56 ± 0.03	0.92 ± 0.01	0.047 ± 0.02	0.57 ± 0.01				

Table 2: Polymorphic Information Content (PIC), Shannon's index, Number of observed and expected alleles, Nei' she Ter zygosity and Wright's fixation index, observed and expected heterozygosity, chi-square and G-square value at duck specific microsatellite loci in crossbred duck (F1) generation.

H_o = Observed heterozygosity; He = Expected heterozygosity; f = Degrees of freedom; ***p ≤ 0.001.

Conclusions

In conclusion, the identified appropriate microsatellite marker systems for crossbred ducks will provide a good choice for genetic monitoring of the quality and the population genetic diversity of poultry stocks.

Author’s Contribution

All the Authors have equally contributed for the research article.

Conflict of Interest

The *authors declare that there is no Conflict of Interests regarding the publication of this article.*

Acknowledgement

The authors are thankful to the Department of Biotechnology, Govt. of India, New Delhi (Project ID 102/IFD/SAN/1335/2018-2019) for financial assistance to carry out the research work.

Bibliography

1. Alyethodi Rafeeque and Kumar Sanjeev. “Genetic Characterization of Moti Indian Native Duck Using Microsatellite Markers”. *Journal of Applied Animal Research* 38.1 (2010): 223.
2. Botstein D., *et al.* “Construction of a genetic linkage map in man using restriction fragment length polymorphisms”. *The American Journal of Human Genetics* 32 (1980): 314-331.
3. Hamilton MB., *et al.* “Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites”. *Biotechniques* 27 (1999): 500-507.
4. Hsu YC., *et al.* “Isolation and characterization of microsatellite DNA markers from the Lanyu scops owl (*Otus elegans botelensis*)”. *Molecular Ecology Notes* 3 (2003): 595-597.
5. Huang YH., *et al.* “Characterization of 35 novel microsatellite DNA markers from the duck (*Anas platyrhynchos*) genome and cross-amplification in other birds”. *Genetics Selection Evolution* 37 (2005): 455-472.
6. Huang YH., *et al.* “A Genetic and Cytogenetic Map for the Duck (*Anas platyrhynchos*)”. *Genetics* 173 (2006): 287-296.
7. Maak S., *et al.* “Isolation and characterization of 18 microsatellites in the Peking duck (*Anas platyrhynchos*) and their application in other waterfowl species”. *Molecular Ecology Notes* 3 (2003): 224-227.
8. Yeh FC., *et al.* “POPGENE Version 1.32: Microsoft Window-Based Freeware for Population Genetics Analysis”. University of Alberta, Edmonton (1999).